ANTIMALARIAL ACTIVITY OF 4(1H)-QUINOLONES

Kyle DE, Gerena L, Pitzer K, Gettyacamin M

Antimalarial activity of 4(1H)-quinolones was first recognized in the 1940's. Endochin [2-methyl-3n-heptyl-7-methoxy-4 (1H)-quinolone] had causal prophylactic and erythrocytic stage activity in avain malaria models, yet was not efficacious against malaria parasites of mammals (Steck, 1972). Subsequent evaluation of coccidiostat quinolone analogs identified ICI 56,780, which was found to have causal prophylactic (single dose of 30 mg/kg sc) and blood schizonticidal activity (ED_{50} = 0.05 mg.kg) in rodent malaria models (Ryley and Peters, 1970). Unfortunately a high degree of resistance to ICI 56,780 was obtained after one passage in *Plasmodium berghei* infected mice and the series was abandoned. The intriguing potency against exoerythrocytic and erythrocytic stages of malaria prompted us to evaluate a series of endochin and ICI 56,780 analogs identified in the WRAIR Chemical Inventory. Interestingly, a series of 2-methyl-3-(1'-alkenyl)- or -3-alkyl-4(1H)-quinolones (Casey, 1974; e.g. WR193211) were remarkably active against erythrocytic stages of multidrug resistant isolates and clones of *P. falciparum in vitro.* WR193211 IC50s ranged from 5.7 - 16.6 nM and no cross resistance was evident with choroquine, quinine, mefloquine, or cycloguanil. In the 3-day Thompson test (*P. berghei* infected mice) WR193211 demonstrated oral activity (SD_{90}= 97 mg/kg). Due to structural similarities to napthoquinones and ubiquinone, we also evaluated the activity of the WR193211 analogs against atovaquone resistant *P. falciparum* and several of the quinolone analogs exhibited strong cross resistance with atovaquone. For example, IC_{50}s for WR193215 ranged from 2.4 - 10.6 nM for atovaquone susceptible parasites and from 348.6 - 3,302 nM for atovaquone resistant *P. falciparum.* In comparison, endochin was extremely effective against a chloroquine susceptible clone (D6 IC_{50}= 12.7 nM), but was 10-fold less effective against an atovaquone-resistant isolate (TM90-C2B IC_{50}= 119.0 nM). In summary, these data demostrate potent erythrocytic stage activity of 4(1H)-quinolones. Cross resistance with atovaquone suggests a similar mechanism of action and may explain the easy selection of ICI 56,780 resistance in *P. berghei.* Importantly, cross-resistance with atovaquone in *P. falciparum* was not complete across the series; therefore, additional structure-activity studies with the class may lead to novel causal prophylactic drug candidates.

American Society of Tropical Medicine and Hygiene 54th Annual Meeting. Washington, DC, USA. 11-15 December 2005. (Poster)

ANTIMALARIAL DRUG RESISTANCE IN ISIOLO, KENYA

Bedno S, Coldren R, Achilla R, Liyala P, Eyase F, Wangui J, Akala H, Mbui J, Waters N

Antimalarial drug resistance is an increasing problem in Kenya with regional variability. With wide spread resistance to the commonly used drugs such as chloroquine and sulfadoxine-pyrimethamine (SP), surveillance projects must be implemented to identify alternative drugs and estimate the burden of disease. Patterns of drug resistance in the semi-arid region of Isiolo have not been previously documented. This region can be classified as mesoendemic for malaria, with most malaria transmission occurring during the long and short rainy seasons. A study of

malaria and drug resistance patterns was undertaken in Isiolo from July 2003 to June 2004 and 133 subjects were enrolled. Blood smears, whole blood, and epidemiological data were obtained from the study volunteers after obtaining informed consent. Analyses included *in vitro* drug sensitivity testing against a panel of 15 antimalarial agents and PCR to assess for genetic polymorphisms. Based on established cut-off values, we found significant resistance of cultured parasites to halofantrine (90%), dapsone (75%), and pyrimethamine (100%). Moderate resistance was also observed to mefloquine (45%) and sulfadoxine (50%). Genetic mutations in malarial genes can result in parasites becoming less sensitive to specific drugs. Additionally, mutations within a single gene can induce cross resistance so that parasites become less sensitive to specific drugs in the absence of any drug pressure. This is most likely the case with mefloquine resistance since this drug is rarely used throughout Kenya. Interestingly, this region appears to be experiencing low resistance to chloroquine (24%). This may be a result from it being replaced by SP as a first-line therapy in 1998 in Kenya and later being discontinued from the market. Additionally, no isolated parasite from Isiolo had mutations at codon 164 of PfDHFR which appears to be the trend throughout East Africa. The results on genotype analyses as well as additional antimalarial drugs with less defined resistance threshold values will be discussed.

American Society of Tropical Medicine and Hygiene 54th Annual Meeting. Washington, DC, USA. 11-15 December 2005. (Poster)

ARTELINATE ADMINISTERED INTRAVENOUSLY TO RHESUS MONKEYS (*MACACA MULATTA*): A NEUROLOGY AND NEUROPATHOLOGY STUDY. I. THE MEDULLA OBLONGATA, PONS, AND CEREBELLUM

Petras JM, Van Gessel Y, Blanchard TW, Miller RS, Bracke KM, Charya AV

Male and female Rhesus monkeys were administered artelinate (AL, artelinic acid): 5.9, 11.8 or 47.2 mg/kg/d x 7d. Vehicle controls were given $NaHCO_3$ while positive controls were given arteether at 16 mg/kg/d x 14d. Neurological deficits were not observed and motor activity was normal in monkeys administered artelinate at doses of 5.9 or 11.8 mg/kg/d x 7d. Monkeys given 11.8 mg/kg of artelinate showed moderately diminished food intake. Neurological signs were present in monkeys given 47.2 mg/kg/d of artelinate. Ataxia was transient (25% of monkeys). Tremors, muscle fasciculations and convulsions were not observed. Motor activity was decreased lasting for 1-3 days during 1-7 days. Salivation and vomiting was observed in 25% of the monkeys. Somnolence was transient and non-progressive (100%). Vehicle control monkeys were neurologically asymptomatic. Foot tremor, arm tremors and piloerection was seen in one AE-treated monkey. Mortality was not observed. Post-treatment survivals were 14 days duration. On day 21 the monkeys were surgically anesthetized, euthanasia by exsanguinations, and transcardial perfusion fixation with Bouin's fluid to preserve the central nervous system for microscopic study. Matching vehicle controls ($NaHCO_3$) were prepared together with arteether positive controls (16 mg/kg/d x 14d for a total dose of 224 mg/kg). The brains of AS-treated, vehicle controls, and AE-treated monkeys were blocked in the horizontal plane. Serial sections of paraffin embedded blocks were cut at 10 μm and stained according to the methods of Nissl,